concentration of FFAs than BTC-6, due to the increased sensitivity of these cells. Mitochondrial function was monitored by measurements of the mitochondrial oxygen consumption (high-resolution respirometry), ATP content (PerkinElmer), mitochondrial membrane potential [flow cytometry (BD)], activity of the caspase-9 (R&D Systems) and ROS generation (DCFH-DA). In preadipocyte cell line: palmitic acid impaired mitochondrial function that was manifested by a decreased intracellular ATP content and an increased ROS generation. Arachidonic acid increased ROS generation and caspase-9 activity related with the mitochondrial apoptosis pathway. Eicosapentaenoic acid decreased mitochondrial respiration and ATP content. In BTC6 line increased the amount of early apoptotic cells after incubation with AA and EPA was observed. There was also a tendency to increased caspase-9 activity after incubation with FFAs. Arachidonic acid slightly increased mitochondrial respiration in beta cells. In conclusion, free fatty acids affect mitochondrial function of selected adipose and pancreatic cell lines which is interesting in the light of a recent data, indicating that regulation of mitochondrial activity may be the target pathway for treating obesity. Supported by K/PBN/000001, K/ZDS/004496.

doi:10.1016/j.bbabio.2014.05.024

S7.P18

Compromised mitochondrial function drives endocrine crosstalk through a distinct protein secretome in muscle

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Systemic energy metabolism requires coordination of different organs to maintain homeostasis and overcome physiological and stress challenges. Skeletal muscle is a major metabolic organ that responds to energy shortage. Here, we show that nutrient deprivation of cultured muscle cells (C2C12) changes their bioenergetic profile. Differentiated myotubes were deprived of glucose and l-glutamine for 30 min up to 24 h. Plate-based respirometry showed that the lack of glycolysis was first compensated by an increased mitochondrial ATP turnover. In the sequelae of nutrient deprivation, mitochondrial respiration and ATP production were progressively compromised until reaching levels below reliable detection after 24 h. After 8 h of nutrient deprivation, respiration linked to ATP synthesis was decreased to 36% of non-fasted controls. Fibroblast Growth Factor 21 (FGF21), previously shown to be induced upon a reduced mitochondrial energy output in muscle mediating endocrine crosstalk between organs, increased mRNA levels by 13-fold and the secretion of FGF21 protein by 5-fold. Under these conditions, we assessed the protein secretome unbiased using mass spectrometric analysis. In the complex secretome, we found the highest released protein to be FGF21, confirming the suitability of our analysis to discover novel players mediating fasting induced systemic crosstalk. We identified a total of 1498 proteins; 93 of these were significantly induced by starvation, of which 21 were previously published to be secreted and thus represent potential myokines. While FGF21 regulates carbohydrate and lipid metabolism, other novel proteins may display similar effects to maintain systemic homeostasis and will be explored in vivo, offering novel therapeutics to treat metabolic diseases.

doi:10.1016/j.bbabio.2014.05.025

S7.P19

Simple or still not discovered TOM complex of the amoeba *Acanthamoeba castellanii*

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The vast majority of mitochondrial proteins are encoded in the nucleus; therefore they have to be imported from the cytosol to their place of destination. The efficiency of their import into mitochondria depends on the wide variety of proteins forming translocases localized in both mitochondrial membranes. One of these translocases is the TOM complex (translocase of the outer membrane) regarded as the main gate into mitochondria for imported proteins. The complex is responsible for decoding of targeting signals, translocation of imported proteins across or into the outer membrane, and their subsequent sorting. Thus, undoubtedly the TOM complex is fundamental for mitochondrial functioning. The subunit organization of the TOM complex has been shown to be characteristic for a given phylogenetic lineage. Beside common subunits the complex may contain subunit(s) that is(are) not present in representatives of other phylogenetic lineages. Till now the complex has been described for representatives of fungi and animals (classified as Opisthokonta in the recent eukaryotic classification system) as well as for plants (Archaeplastida). However, for representatives of Amoebozoa, Chromalveolata, Excavata and Rhizaria (former Protista) the amount of data concerning the complex is indeed small. Therefore we decided to study the TOM complex of the amoeba *Acanthamoeba castellanii* classified as a representative of Amoebozoa. This organism is also quaint from the bioenergetics point of view because its mitochondria share many features with both plant and animal mitochondria. Consequently, studies of the amoeba TOM complex may contribute to better understanding of evolution of mitochondria protein import machinery. Here we report isolation of *A. castellanii* functional TOM complex by affinity chromatography using antibody directed against *A. castellanii* Tom40. Apparent molecular mass of the complex determined by native protein electrophoresis is 450 kDa. The channel activity estimated for the complex supports its identification as the TOM complex. The complex subunits, identified due to application of protein electrophoresis and mass spectrometry, correspond to Tom40, Tom7 and Tom20. Thus, the isolated complex can be used for verification of the presence of other subunits indicated by *A. castellanii* transcriptome analysis. The studies were supported by NCN N N303 143937.

doi:10.1016/j.bbabio.2014.05.026